I, Gregor Sagner, declare as follows:

1. I hold the position of director R&D with Roche Applied Sciences, Penzberg, Germany. I have held this position for 7 years. Prior to my present position, after having finished my PhD thesis I worked as researcher in the Molecular Biology R&D department of Roche Applied Sciences for 10 years.

2. I was awarded a PhD degree from the University of Erlangen, Germany in 1988. I have been awarded 7 patents, and have published ~12 articles in peer-reviewed journals.

3. I have read and understood the specification and claims of US Patent Application 10/087,082, entitled "Method of Primer Extension Preamplification PCR". I have also read and understood the US PTO Office Action mailed May 17, 2005 in this application, as well as the references Eggeling et al. (Hum. Genet. 1997 99:266-270) and the Boehringer-Mannheim Biochemicals Catalog (1997), page 153, which are cited therein against the '082 application. I understand that the applicant in this case will amend claims 1 and 9 to replace the phrase "wherein said nucleic acid fragments are between 100 and 1000 base pairs in length" with "wherein said nucleic acid fragments are between 100 and 550 base pairs in length". I am not named as an inventor on this application, nor do I have any legal interest in this application.

4. I understand that it is the position of the Patent Office that Eggeling et al. teaches a method for the amplification of nucleic acid fragments from a sample comprising a pool of cDNAs, wherein the nucleic acid fragments are between 100 and 1000 base pairs; that the method comprises first and second amplification steps, wherein the first amplification is performed using completely randomized primers and the second amplification is performed using specific primers; and that for the first amplification steps, the temperature at which primer extension is performed increases in at least some of the successive amplification cycles. I further understand that it is the position of the

Patent Office that Eggeling et al. does not teach the use of a mixture of at least two thermostable DNA polymerases comprising at least one DNA polymerase without 3'-5' exonuclease activity and a DNA polymerase with 3'-5' exonuclease activity.

5. I understand it is the position of the Patent Office that the Boehringer-Mannheim Biochemicals Catalog teaches use of a mixture of at least two thermostable DNA polymerases for amplification of nucleic acid fragments up to 6 kb (the Expand system); that the mixture comprises a DNA polymerase without 3'-5' exonuclease activity (Taq DNA polymerase) and a DNA polymerase with 3'-5' exonuclease activity(Pwo DNA polymerase). I further understand that it is the position of the Patent Office that it would have been obvious to one of skill in the art to substitute the DNA polymerase mixture of the B-M Biochemicals Catalog in the method of Eggeling et al. to achieve a sensitive method of amplification, because the Catalog teaches the use of the DNA polymerase mixture reduces secondary structure and the error rate, thus providing a high fidelity PCR system capable of amplifying entire populations of RNA transcripts without the need to construct cDNA libraries. I further understand that it is the position of the Patent Office that the person of skill in the art would have been motivated to make such a substitution in attempting to achieve a increased fidelity during primer extension, and that they would have had a reasonable expectation of success in doing so.

6. It would not have been obvious to substitute the Expand system in the method of Eggeling et al., because the Expand system, as can be deduced from the catalog, is intended to be used for the amplification of long (>1000 bp) templates ("...and the Expand™ PCR System's unique blend of thermostable enzymes lead to greater fidelity (low error rate) *and larger fragments (up to 6 kb)*". It is known in the art that amplification of PCR fragments of about 550 bp or smaller can be generated with almost 100% fidelity, such that no 3'-5' proofreading activity is required. Therefore, one of skill in the art would be concerned about increased fidelity, and the beneficial effects of 3'-5'

proofreading activity thereon, only when amplification of PCR fragments greater than about 500 bp is desired.

7. Eggerling et al.use Taq DNA polymerase for both random and specific amplification steps. Eggerling et al. disclose the generation of 0.2-0.5 amplification products for the first round of amplification, i.e. the whole genome amplification with random primers. For the second round of specific amplification, they disclose amplification of microsatellite loci without mentioning the size (these loci are typically below 100 bp) and amplification of the ameliogenin gene resulting in 106/112 bp amplification products. Consequently, Eggerling et al. provides no motivation to replace the Taq polymerase with an enzyme mixture comprising a DNA polymerase with 3'-5' proofreading activity, simply because there is no reason why one of skill in the art would believe that such an activity would theoretically increase the performance of the method disclosed by Eggerling et al.

Oct 20, 2005

Date

Dr. Gregor Sagner